



Fluid dynamic constraints on resource acquisition in small pelagic organisms

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RESEARCH ARTICLE

Stress and food deprivation: linking physiological state to migration success in a teleost fish

Jonathan D. Midwood^{1,*}, Martin H. Larsen², Kim Aarestrup² and Steven J. Cooke¹

ABSTRACT

Food deprivation is a naturally occurring stressor that is thought to influence the ultimate life-history strategy of individuals. Little is known about how food deprivation interacts with other stressors to influence migration success. European populations of brown trout (*Salmo trutta*) exhibit partial migration, whereby a portion of the population smoltifies and migrates to the ocean, and the rest remain in their natal stream. This distinct, natural dichotomy of life-history strategies provides an excellent opportunity to explore the roles of energetic state (as affected by food deprivation) and activation of the glucocorticoid stress response in determining life-history strategy and survival of a migratory species. Using an experimental approach, the relative influences of short-term food deprivation and experimental cortisol elevation (i.e. intra-coelomic injection of cortisol suspended in cocoa butter) on migratory status, survival and growth of juvenile brown trout relative to a control were evaluated. Fewer fish migrated in both the food deprivation and cortisol treatments; however, migration of fish in cortisol and control treatments occurred at the same time while that of fish in the food deprivation treatment was delayed for approximately 1 week. A significantly greater proportion of trout in the food deprivation treatment remained in their natal stream, but unlike the cortisol treatment, there were no long-term negative effects of food deprivation on growth, relative to the control. Overall survival rates were comparable between the food deprivation and control treatments, but significantly lower for fish in the cortisol treatment. Food availability and individual energetic state appear to dictate the future life-history strategy (migrate or remain resident) of juvenile salmonids while experimental elevation of the stress hormone cortisol causes impaired growth and reduced survival of both resident and migratory individuals.

KEY WORDS: Glucocorticoid, Stress, Starvation, Passive integrated transponder tags, Freshwater, Brown trout

INTRODUCTION

For decades, researchers have conducted laboratory studies on the effects of different stressors on the physiology, condition, behaviour and survival of various animals. Such research has formed the basis for major research areas such as comparative physiology (Mangum and Hochachka, 1998) and environmental physiology (Willmer et al., 2009), and has helped to shape paradigms related to how organisms respond to different stressors. Although this foundational

work is critical, animals in the wild may perceive and respond to stressors very differently than they do in captivity. This has led to the genesis of ecological physiology (Feder and Block, 1991) and broad calls for reinvigorating comparative physiology through field experimentation (e.g. Mangum and Hochachka, 1998; Somero, 2000). Fundamental to ecological physiology is the need to include ecologically relevant endpoints such as survival and reproduction (Pough, 1989; Gilmour et al., 2005) in an attempt to understand the ecological implications of physiological variation (Spicer and Gaston, 1999) and responses to stressors (Pankhurst, 2011; Boonstra, 2013a). Research in the field is both complex and challenging (Costa and Sinervo, 2004), yet, at the same time, provides the ecological relevance needed to understand how physiological state (e.g. stress) of individuals may explain population-level and evolutionary processes (Calow and Forbes, 1998; Ricklefs and Wikelski, 2002).

The neuroendocrine glucocorticoid (GC) stress response in vertebrates (see Sapolsky et al., 2000) represents an example of a system and response that was long-studied in the laboratory and has only recently been explored in field settings. Through analysis of tissue samples intended to characterize baseline or stress-induced GC levels (see Dantzer et al., 2014) to various GC manipulation studies (reviewed in Sopinka et al., 2015; Crossin et al., 2016), researchers have started to elucidate what is now termed ‘the ecology of stress’ (Boonstra, 2013a). Yet, many challenges remain, as studies often use GC manipulations to simulate semi-chronic or chronic stressors that may not be ecologically relevant (Crossin et al., 2016; Sopinka et al., 2015). Natural experiments, where natural processes are re-created directly in the field, avoid this type of issue (i.e. predation: Sheriff et al., 2011; thermal stress: Quigley and Hinch, 2006; flow reduction: Krimmer et al., 2011), but can be challenging to implement on a large scale in the field while standardizing stress exposure for each individual. While artificial manipulations are often intended to mimic natural phenomena such as storms or other extreme weather events (Romero et al., 2000; Pankhurst, 2011; Wingfield, 2013), studies that characterize the stress of predation, competition or starvation in the wild are relatively uncommon (Boonstra, 2013b). Relatedly, there is a need to move beyond being simply focused on physiological endpoints to include those that incorporate behaviour and more closely approximate fitness-related factors. This will result in a more complete understanding of the ecology of stress (Boonstra, 2013a).

Food deprivation is a ubiquitous natural phenomenon that occurs when a post-absorptive animal, otherwise willing or able to eat, is unable to do so as a result of some extrinsic limitation on food resources (McCue, 2010). Periods of limited food intake (for various durations ranging from hours to months) due to spatial and temporal heterogeneity of food resources are common in wild animals, limiting population size and biological productivity (McNamara and Houston, 1987). When animals are exposed to periods of reduced food intake, simple bioenergetics principles

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related to the balance between consumption and expenditure would suggest that energetic conditions would decline, with limited energy that could be devoted to growth or reproduction (Kleiber, 1961; McCue, 2010). Indeed, a continuing supply of energy is necessary for an animal to live given that even the most basic physiological processes have an energy cost (Porter and Gates, 1969).

When exposed to food deprivation, particularly for lengthy bouts, declines in nutritional condition (which can extend beyond macronutrients to include vitamins and minerals; Halver and Hardy, 2002) may lead to impairments in immune function (e.g. Caruso et al., 2011), induce oxidative stress (Pascual et al., 2003), and alter general health (Wang et al., 2006) and even behaviour (e.g. malaise). In extreme cases, food deprivation can lead to mortality – either directly or indirectly – close to when the food deprivation period occurs (i.e. when there is insufficient energy to maintain homeostasis; McCue, 2010) or at a future time (i.e. a carry-over effect; Harrison et al., 2011; O'Connor et al., 2014). However, given that food limitations are common in the wild, it is not surprising that fish have a variety of adaptive biochemical, physiological and behavioural responses to maximize survival (Wang et al., 2006; McCue, 2010).

In teleost fishes, activation of the hypothalamic-pituitary–interrenal (HPI) axis and production of cortisol mobilizes energy that may allow the individual to survive a stressor (reviewed in Mommsen et al., 1999). This activation typically occurs for a short duration and therefore activation of the HPI axis is more often acute than chronic. Continuous activation of the HPI axis results in continued mobilization of energy, reducing growth, disrupting immune function and preventing the creation of lipid reserves (Espelid et al., 1996; Gregory and Wood, 1999; Mommsen et al., 1999; Crespi et al., 2013). These negative consequences are similar to issues associated with extended food deprivation; however, through activation of the HPI axis, these will occur simultaneously rather than as a result of food deprivation.

Using wild juvenile brown trout (*Salmo trutta* Linnaeus 1758) as a vertebrate teleost model, we sought to study the ecology of stress in a natural stream system. Specifically, we tested the hypothesis that a natural stressor influences growth rate, survival and life-history strategy of juvenile brown trout. To mimic a natural stressor, we exposed fish to a 14 day food deprivation protocol while holding fish in food-limited enclosures in the stream. Food deprivation is particularly relevant to juvenile salmonids, such as brown trout, as low energy stores are associated with poor growth, survival and their ultimate life-history strategy (Forseth et al., 1999). Further, we tested the hypothesis that exogenous cortisol manipulation (using corticosterone embedded in a cocoa butter carrier – see Gamperl et al., 1994) has a similar influence to the more ‘natural’ food deprivation stressor.

Brown trout were chosen as a model for several reasons. From a logistical perspective, the juveniles reside in small streams, which enables fish to be collected via electrofishing and makes it easier to track individual fish (for survival and behaviour, and – if recaptured – for growth and condition) using small passive integrated transponder (PIT) tags (Gibbons and Andrews, 2004). Moreover, the brown trout in the present study exhibit an interesting life-history strategy in that they perform partial migration (Jonsson and Jonsson, 1993; Alerstam et al., 2003), wherein some juveniles smoltify and move downstream to the ocean to feed while others remain in the stream, forming resident populations of typically smaller fish.

MATERIALS AND METHODS

Study site

Gudlø Stream, located in central-eastern Jutland, Denmark, supports a wild population of partially anadromous brown trout

(Fig. 1). This stream, and its connecting tributaries, runs for over 16 km before entering the western Baltic Sea at Kolding Fjord. In general, the stream is shallow (<1.0 m) and less than 2.0 m in width. Approximately 1 km upstream of this connection, two PIT reading stations continuously log the passage of tagged fish. Station 1 (S1) consists of two antenna spaced 10 m apart and is situated approximately 150 m upstream of station 2 (S2), which also consists of two antenna. This paired configuration allows determination of the direction of movement of tagged trout. Detection efficiency at S1 was estimated as 98.5%, calculated as the percentage of trout that were known to have passed S1 (i.e. detected at S2) that were actually detected at S1 (after Zydlewski et al., 2006). In the short distance between S1 and S2, the stream flows through a millpond, with a small fish ladder at its outflow just upstream of S2. This millpond has previously been identified as a bottleneck that limits downstream migration for brown trout in the system (Midwood et al., 2014, 2015); passage at S2 is therefore expected to be naturally lower than that at S1.

Capture and treatment

Animal care approval for this study falls under the Danish Animal Experiment Inspectorate (licence number: 2013-15-2934-00808). On 28 February and 1 March 2013, four sections of Gudlø Stream were sampled using single-pass backpack electroshocking (Scubla ELT 60 II G, running at 300 V). In each section, between 46 and 53 brown trout greater than 12 cm in total length were collected, giving a total of 202 individuals. The total length (cm) and wet mass (g) of each individual was recorded and a uniquely coded 23 mm PIT tag (RI-TRP-RRHP, 134 kHz, 0.6 g mass in air, Texas Instruments, Plano, TX, USA) was inserted into the body cavity following methods outlined in Midwood et al. (2014). A similar approach in the con-generic Atlantic salmon (*Salmo salar*) was found to have high rates of tag retention and survival (Larsen et al., 2013). Trout

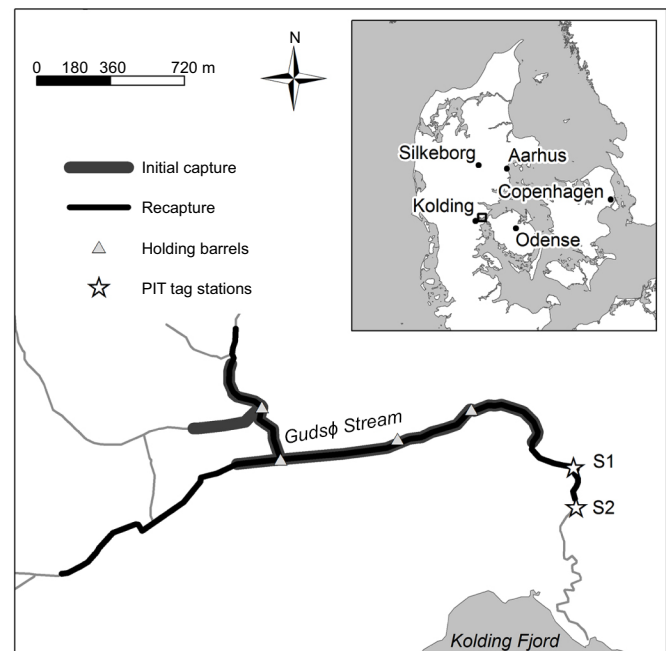


Fig. 1. Study location. Gudlø Stream is situated northeast of Kolding in Jutland, Denmark. The portions of the stream where brown trout were initially captured in spring 2013 and where they were recaptured in June 2013 are shown. The four holding locations for the food deprivation containers and the location of the PIT reading stations (S1 and S2) are also shown.

were then placed into one of four 100 l barrels with approximately 50 individuals in each, which were subsequently secured in the stream near to their capture site (Fig. 1). These barrels had 1.0 cm holes drilled across their surface to allow stream water to flow through, but prevent the trout from escaping. Limited shelter was available within the barrels in the form of large stones used to weigh the barrel down in the stream. Trout were kept in these barrels for 14 days to simulate a 2 week food deprivation period. Prior to their release on 14 and 15 March 2013, the individual total length and wet mass (minus 0.6 g to account for the PIT tag) of trout in this ‘food deprivation’ treatment (herein FD) were again assessed to determine whether there were changes as a result of the holding period.

Also on 14 and 15 March 2013, an additional 421 trout were collected from five sections of Gudsø Stream, which overlapped the areas where trout in the FD treatment were captured (Fig. 1). The total length and wet mass were measured for all of these additional trout and they were PIT tagged in the same manner as for fish in the FD treatment. After tagging, these trout were assigned to one of two groups using a stratified random approach to ensure approximately equal sample sizes and size distributions. The first group (209 in total) was assigned to the control treatment and fish were released following their recovery. The remaining 211 trout were assigned to a cortisol treatment, where each individual received an intra-coelomic injection of a suspension of cocoa butter (100% pure cocoa butter, Now Foods, Bloomingdale, IL, USA) and hydrocortisone 21-hemisuccinate (Sigma-Aldrich, product no. H2882-1G) at a dosage of 100 mg kg⁻¹. A recent validation study carried out under natural conditions found that this treatment raised circulating plasma cortisol levels in brown trout to over 200 ng ml⁻¹ (K. Birnie-Gauvin, K. S. Peiman, M.H.L., K.A., K. M. Gilmour and S.J.C., unpublished data), which is above documented levels for an acute handling stressor (130 ng ml⁻¹; Pickering et al., 1982) and considerably more than what has previously been reported in a similar laboratory-based validation study (20–40 ng ml⁻¹; Pickering, 1989). These elevated levels persisted for at least 3 days, but had returned to baseline conditions (equal to a control and sham treatment) after 6 days. Consequently, this treatment is consistent with a semi-chronic stressor (i.e. longer than acute but not particularly prolonged such that it would be chronic). Sham treatments were not included in the present study, but previous work on brown trout has suggested that, relative to a control, treatment with cocoa butter alone reduced growth rate (length), but did not affect survival (Midwood et al., 2014). Circulating plasma cortisol levels in sham-treated brown trout were also not found to

differ significantly from a control (K. Birnie-Gauvin, K. S. Peiman, M.H.L., K.A., K. M. Gilmour and S.J.C., unpublished data).

On 18 and 19 June 2013, five sections of the stream were re-sampled using single-pass backpack electrofishing to capture trout that did not migrate and instead had become resident in the stream (Fig. 1). Previous estimates of brown trout capture efficiency with this technique range between 52% and 90% (Buttiker, 1992) and are typically higher in narrow shallow systems like Gudsø Stream as brown trout are actively drawn to the anode. All trout were scanned (Agrident, APR350) to determine their PIT tag number, and their length and wet mass were measured. For the recaptured trout, the instantaneous growth rate for both length (G_L) and mass (G_M) was calculated according to Eqn 1 (after Schreck and Moyle, 1990):

$$G = (\log_e Y_2 - \log_e Y_1) / (t_2 - t_1), \quad (1)$$

where Y_1 is the length or mass at the time of tagging (t_1) and Y_2 is the length or mass at the time of recapture (t_2). The length and mass for trout at the time of their release was used for Y_1 . Therefore, the length and mass of FD trout after the 2 week holding period was used for Y_1 . A relative condition factor (K_R ; after Le Cren, 1951) was developed for the sample population based on the relationship between the log-transformed length and mass. The K_R for each individual was then calculated based on Eqn 2:

$$K_R = \log(\text{wet mass}) / [-1.84 + 2.81 \log(\text{length})]. \quad (2)$$

Statistical analysis

A paired *t*-test was used to determine the extent of the changes in mass and K_R for trout in the FD treatment following the 2 week holding period. Product-limit log-rank survival analyses were conducted to determine whether the number of detections at S1 and S2 differed among the three treatments. Logistic regression was used to compare the relative proportions of trout in each treatment that were recaptured in the stream or were known to survive (either recaptured or detected passing S1 or S2).

ANOVA was used to compare G_L and G_M among treatment groups. Similarly, ANOVA was also used to compare the mean number of days it took trout in each treatment group to pass S1. When significant, a *post hoc* Tukey HSD was conducted to determine which treatments differed. All analyses were completed in JMP 9.0 (SAS Institute, Cary, NC, USA) with alpha evaluated at $P=0.05$.

Table 1. Mean (±s.d.) initial length, mass and relative condition (K_R) for trout in each of the three treatment groups

Metric	Treatment		
	Control	Cortisol	Food deprivation
Sample size	209	211	202
Length (cm)	14.3±1.4 (12.0–19.7)	14.3±1.6 (12.0–22.8)	14.4±1.5 (12.0–19.8)
Mass (g)	26.6±8.4 (14.6–80.5)	26.7±10.2 (14.9–106.6)	27.5±9.1 (12.1–65.7)
K_R	1.00±0.02 (0.95–1.06)	1.00±0.02 (0.94–1.10)	1.00±0.02 (0.90–1.05)
Days to S1 passage	36.5±17.7 ^{a,b}	35.4±16.0 ^a	42.4±15.0 ^b
No. passing S1	99	78	71
No. passing S2	80	61	61
No. recaptured	21	18	41
Recaptured G_L	0.002±0.001	0.001±0.001	0.002±0.0005
Recaptured G_M	0.008±0.002	0.005±0.002	0.008±0.002

The ranges (minimum–maximum) for each metric are shown in parentheses. The mean number of days between release and passage to S1 is presented; superscript letters show means that are significantly different (ANOVA and Tukey HSD). The total number of trout that were detected passing S1 and S2 and the number of trout that were recaptured in the stream during June surveys is also shown. Finally, the mean (±s.d.) instantaneous growth rates for length and mass for recaptured trout are shown.

Table 2. Output from the log-rank survival analysis

Station	Treatments compared	χ^2	P-value
S1	Control–cortisol	4.62	0.032
	Control–food deprivation	7.98	0.005
	Cortisol–food deprivation	0.42	0.515
S2	Control–cortisol	4.75	0.029
	Control–food deprivation	4.02	0.050
	Cortisol–food deprivation	0.04	0.849

For all analyses, the degrees of freedom was 1 and alpha was set to $P=0.05$.

RESULTS

In total, 622 trout were captured, tagged and treated, with similar initial length, wet mass and K_R among treatments (Table 1). There was a significant decline in mass for trout in the FD treatment following the 2 week holding period ($t_{201}=-11.54$, $P<0.0001$) with an average of 1.02 ± 1.26 g of mass lost [$3.8\pm2.8\%$ of their initial biomass, ranging from a maximum loss of 4.2 g (-13.8%) to a net gain of 1.2 g ($+4.9\%$)]. There was also a significant concomitant decline in K_R following the holding period ($t_{201}=-237.23$, $P<0.0001$, mean difference= -0.433 ± 0.026).

For all treatments, less than half of all tagged fish were detected passing S1 and S2. The proportion passing the stations was lower for both the FD and cortisol treatments relative to the control (Table 1). This was confirmed with the survival analysis, with significantly lower passage of both food-deprived and cortisol-treated trout relative to the control at both S1 and S2 (Table 2). There were no differences, however, in survival to either station between the FD and cortisol treatments. While it is clear that there was reduced passage at both stations for the FD and cortisol treatments (Fig. 2), passage of fish in the FD treatment at S1 also took significantly longer (ANOVA, $F_2=3.88$, $P=0.022$; Table 1) than that of fish in the cortisol treatment (Tukey HSD, $P=0.028$). Although not significant (Tukey HSD, $P=0.057$), there was a trend towards fish in the FD treatment taking longer than those in the control treatment. Indeed, passage by fish in the FD treatment took 6 and 7 days longer than that by fish in the control and cortisol treatments, respectively (Table 1).

A total of 80 trout were recaptured during the June surveys. There was a significantly greater proportion of trout from the FD treatment (0.20) recaptured during these surveys relative to the control (0.10) and cortisol treatments (0.08; $\chi^2_{2}=13.989$, $P=0.0009$; Table 1, Fig. 3). In contrast, there was a significantly smaller proportion of trout from the cortisol treatment that were known to survive (i.e. recaptured or detected at S1 or S2) and no differences between the control and FD treatments ($\chi^2_{2}=6.675$, $P=0.0355$; Fig. 3). There were also significant differences among treatments for both G_L and G_M (ANOVA, $F_2=1.6094$, $P<0.0001$ and $F_2=16.1997$, $P<0.0001$, respectively). For both instantaneous growth rates, values for the cortisol treatment were significantly lower relative to those of both the control and FD treatments (Tukey HSD, $P<0.001$). There were no differences, however, between the control and FD treatments for either G_L or G_M (Tukey HSD, $P>0.8$).

DISCUSSION

For fish, and indeed many invertebrates, food deprivation represents a common natural stressor associated with spatio-temporal variation in the abundance of appropriate food items. In the present study, we exposed fish to a 14 day period of food restriction, which, given the cool water temperatures, would presumably result in modest food deprivation and declines in energetic condition (e.g. Byström et al., 2006). Fish were held in the river in barrels with holes such that some natural forage would occasionally pass through the barrels, although much less than would be available to them if they were able to move about freely and had access to smaller-bodied fish, terrestrial invertebrates and benthic invertebrates. On average, fish lost 3.8% of their initial mass, although a few individuals gained a small amount of mass (up to 4.9%) or maintained their initial mass, while several fish exhibited extreme loss of mass (up to 13.8%). Overall, there was a general decline in body condition and negative growth. The fact that we had variable responses to food limitation would not be unexpected given individual differences in behaviour, physiology and genotype (Koolhass et al., 1999; Adriaenssens and Johnsson, 2011, 2013). The food limitation we imposed on the fish occurred during a life-history period where food is exceedingly

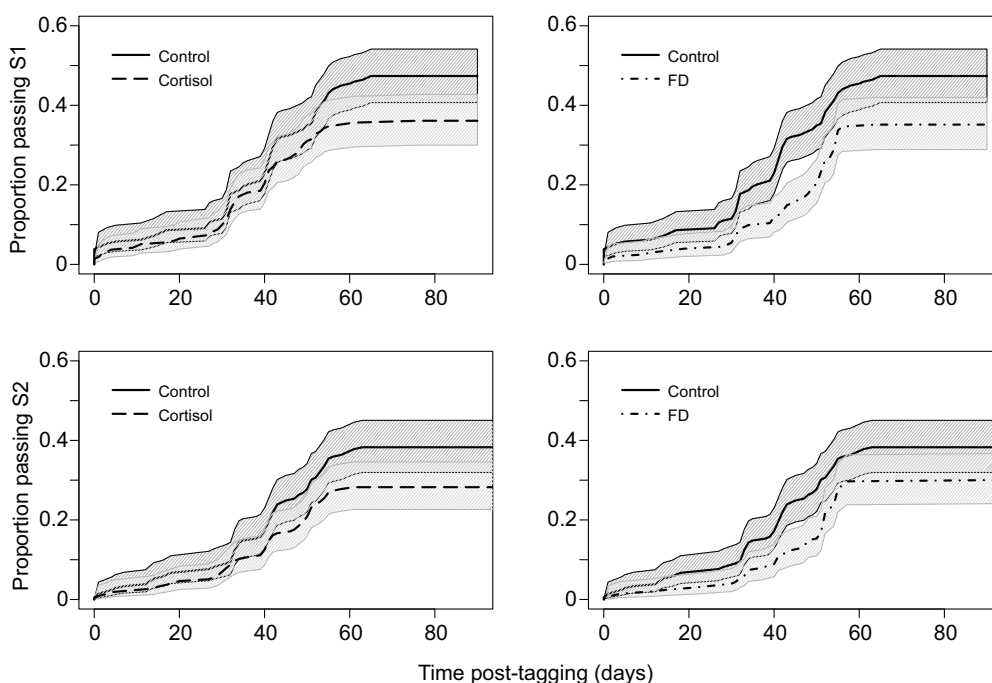


Fig. 2. Visualization of the output from the product-limit log-rank survival analyses. The top two panels are for brown trout passage at S1 [control $N=99$, cortisol $N=78$ and food deprivation (FD) $N=71$] and the bottom two panels are for passage at S2 (control $N=80$, cortisol $N=61$ and FD $N=61$). The shaded areas show the 95% confidence intervals for each treatment.

important. Juvenile brown trout feed extensively while in streams to prepare for migration; therefore, even small reductions in food intake during early life stages of salmonids can be deleterious (Jonsson and Jonsson, 1998). Conversely, increases in food availability (e.g. through supplemental feeding) can improve survival and stream carrying capacity (Mason, 1976). When exposed to transient periods of food deprivation, compensatory growth is possible (Nicieza and Metcalfe, 1997), but negatively influences survival in the future (Johnsson and Bohlin, 2006).

Unfortunately, we cannot completely discount that trout held as part of the FD treatment were not also stressed as a result of being in tanks in somewhat crowded conditions. Pickering and Stewart (1984) explored the effects of crowding on cortisol and growth in brown trout and found elevated cortisol levels in brown trout kept in crowded (~1 fish per litre) but not in non-crowded (~0.1 fish per litre) tanks. Growth was also suppressed in the more crowded tank, but this was attributed more to competition for food than to activation of the HPI axis, as the growth impairments continued for the duration of the study, despite cortisol levels returning to baseline conditions within 39 days (study lasted for 110 days; Pickering and Stewart, 1984). In the present study, densities were intermediate between these two treatments (~0.5 fish per litre); therefore, while the FD trout probably experienced an increase in circulating cortisol, it was well below what fish in the cortisol treatment experienced. Consequently, as in Pickering and Stewart (1984), we are confident that the FD treatment rather than a moderate crowding stressor caused the decline in mass.

In this study, we also manipulated cortisol titres of fish experimentally using exogenous cortisol implants. We did so to test whether the semi-chronic cortisol manipulation had a similar influence to the more ‘natural’ food deprivation stressor. In terms of growth, brown trout in the cortisol treatment had significantly lower instantaneous growth rates for both length and mass relative to fish in the control and FD treatments, which is consistent with previous

exogenous manipulations of cortisol in this species (Midwood et al., 2014, 2015) and other teleost fishes (e.g. rainbow trout, *Oncorhynchus mykiss*; Gregory and Wood, 1999). In a similar manner to the FD treatment, reduced food intake in the cortisol treatment probably contributed to a reduction in growth; however, instead of having restricted access to food, increased plasma cortisol levels can suppress a fish’s appetite (Andersen et al., 1991; Gregory and Wood, 1999). Furthermore, even if the cortisol-treated fish consumed food, the additional consequences of activation of the HPI axis, including increased metabolic rate and a reduction in the efficiency of food absorption, would further impair growth and development as well as survival (Barton et al., 1987; Metcalfe et al., 1995; Gregory and Wood, 1999). In contrast, when fish in the FD treatment were released back into the stream, they were free to consume food *ad libitum*, allowing compensatory growth. An important caveat to this conclusion, however, is that unlike previous work (e.g. Gregory and Wood, 1999), a sham treatment was not included in the present study. We therefore cannot conclusively state that the observed reduction in growth rate in the cortisol treatment was solely caused by increased circulating cortisol. Indeed, the vector itself (cocoa butter) may have partially affected growth, as has been documented in previous studies (Hoogenboom et al., 2011; Midwood et al., 2014). Hoogenboom et al. (2011) suggest that a vector may trigger an immune response that increases the basal metabolic rate and consequently reduces growth. While we cannot completely discount this possibility, the exogenous cortisol manipulation used in the present study has been shown to elevate plasma cortisol levels above those of a sham treatment (K. Birnie-Gauvin, K. S. Peiman, M.H.L., K.A., K. M. Gilmour and S.J.C., unpublished data). As a result, while cortisol-treated fish were likely to be affected by the well-documented growth impairments associated with activation of the HPI axis, their growth may have been further impaired as a result of an elevated immune response. Further research, however, is warranted to assess the independent effects of the vector and exogenous cortisol manipulation on growth.

Food deprivation generally leads to elevations in GC levels in salmonid fishes (Barton et al., 1988; Barton and Iwama, 1991; Barton, 2002). Moreover, baseline cortisol levels tend to increase close to smoltification, with a concomitant increase in stress responsiveness during that period (Barton et al., 1985). Relative to control fish, individuals that were food deprived or exposed to cortisol exhibited altered migratory behaviour. The survival analysis revealed significantly lower passage of both food-deprived and cortisol-treated trout relative to the control at both downstream antennas. Moreover, for the FD treatment, there was a clear delay (6–7 days) in the timing of their downstream passage. As brown trout tend to migrate with high spring flows (Bohlin et al., 1993; Aarestrup et al., 2002), this delay for fish in the FD treatment may mean they miss these flood events. Indeed, around day 40 of the study, there was an increase in the number of control and cortisol-treated fish that migrated, but no similar ‘spike’ in the number of FD treatment fish that did so (Fig. 2). It is possible that the fish that were starved were still attempting to compensate for the period of food deprivation such that they would have the nutritional resources necessary to smoltify, migrate and make the transition to life in marine waters. Smoltification is energetically costly (Folmar and Dickhoff, 1980), with lipid metabolism playing a number of critical roles (Sheridan, 1989).

Brown trout exhibit partial migration such that a component of the population becomes resident. In this study, we observed that significantly more fish in the FD treatment became resident.

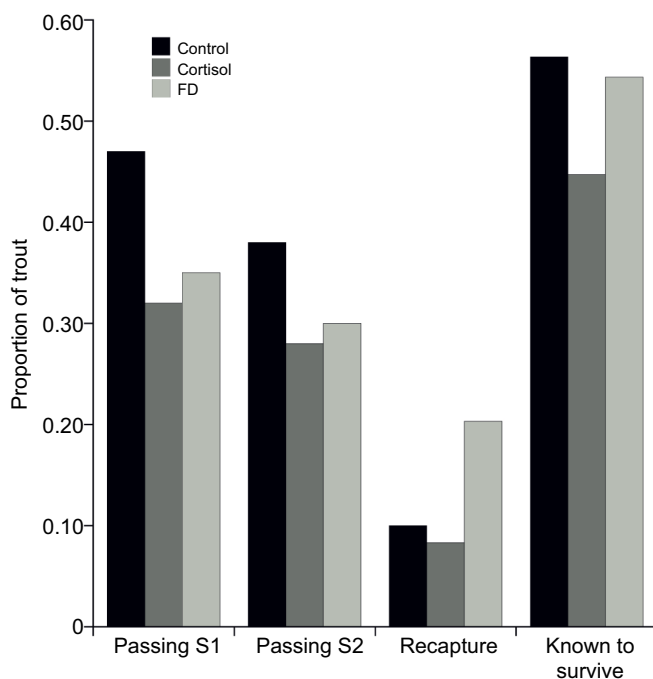


Fig. 3. Trout monitoring. Proportion of trout in each treatment that passed S1 and S2, were recaptured during the June surveys, or were known to have survived (recaptured or detected at either S1 or S2).

Interestingly, an equal number of food-deprived and control trout were known to survive (based on recapture and PIT antennas). In contrast, there were fewer cortisol-treated trout in both the migrant and resident groups. These findings suggest that fish in the FD treatment group did not have reduced mortality but rather their propensity to migrate was reduced. The idea that food deprivation could influence life-history strategy is not surprising given that life history is closely linked to energetics and the endocrine system (Ricklefs and Wikelski, 2002). There is a reasonably large body of literature on the physiological and energetic correlates of partial migration. For example, fast-growing individuals will be constrained by food limitations in a habitat more rapidly than slow-growing individuals and therefore exhibit an increased propensity to migrate in search of additional foraging opportunities (Jonsson and Jonsson, 1993; Chapman et al., 2011; Boel et al., 2014). Furthermore, as migration is associated with increased energy expenditure, individual energetic state may balance the migratory decision (Chapman et al., 2011).

Overall, we found that a natural stressor (food deprivation) and a more experimental stressor (exogenous cortisol manipulation) resulted in alterations in migration behaviour and/or survival relative to control fish. However, there were notable differences in growth rates for cortisol-treated fish as well as in the life-history decision to either migrate or become resident for food-deprived fish relative to controls and cortisol-treated fish. The lack of concordance in rates of partial migration between food-deprived and cortisol-treated fish is itself interesting as both treatments reduce energy and nutritional states and therefore should similarly influence the propensity of an individual to migrate (Chapman et al., 2011). Despite this apparent discrepancy, our previous work has found no difference in migration timing (Midwood et al., 2014) or in migration propensity (Midwood et al., 2015) for cortisol-treated fish relative to a control. It is evident, therefore, that the additional effects of higher plasma cortisol levels that have previously been noted (e.g. increased metabolic rate, reduced gut absorption, impaired immune function, etc.) increased mortality regardless of an individual's migration strategy, but failed to reduce their propensity to migrate. In contrast, food deprivation has been found to impede swimming performance in some fish species (i.e. grass carp, *Ctenopharyngodon idellus*; Cai et al., 2014), while the stress response does not (Gregory and Wood, 1999). This may be a possible explanation for the apparent differences in rates of partial migration; therefore, a focused evaluation of the swimming performance and energetics of individuals from these treatments is warranted.

There is increasing interest in experimentally manipulating GCs in wild animals (Sopinka et al., 2015; Crossin et al., 2016) to simulate different natural and anthropogenic stressors. To our knowledge, this is one of the first studies to directly compare and contrast the consequences of a natural challenge (i.e. starvation via food deprivation) with exogenous cortisol manipulation via implants. Although the consequences of these manipulations were not consistent with respect to partial migration, they did result in similar levels of mortality. Given the potential to use GC manipulations to understand how animals respond to natural and anthropogenic stressors (Sopinka et al., 2015), including novel ones, and explore difficult-to-study phenomena like carry-over effects (O'Connor et al., 2014), we submit that more studies of this nature are warranted. We also encourage future studies that simultaneously manipulate food intake and cortisol (*sensu* Small et al., 2006) on fish with different levels of initial energy density and nutritional status to try to understand the relative roles of different mechanisms on the responses of wild fish to stress.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

J.D.M., M.H.L., K.A. and S.J.C. all helped to design the study. J.D.M. and M.H.L. completed the necessary fieldwork and compiled the data. J.D.M. completed the statistical analyses and J.D.M. and S.J.C. wrote the initial draft of the manuscript. All authors reviewed and revised the manuscript prior to submission.

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